$m/8$ NaCl solution; that the muscle also begins to swell after some time in a neutral hypertonic NaCl solution, while it shrinks in a sufficiently hypertonic NaCl solution if the latter is rendered acid. He ventured the suggestion that this might be a protein reaction.[4] This suggestion has since been amply corroborated by the work of Hardy, Procter and Pauli. It was, moreover, found that this antagonism between acid and salt is much stronger for the system H_2SO_4 — Na_2SO_4 than for the system HCl — NaCl.[5]

These data were utilized to find out whether the specific impermeability of the membrane of the egg of *Fundulus* is due to lipoids or to proteins. It was found that when eggs are exposed to a $N/333$ solution of acetic acid for twenty minutes, their permeability increases to such an extent, that if they are put into a mixture of 50 c.c. 3 *m* NaCl + 1 c.c. 2 1/2 *m* $CaCl_2$ they sink in less than seven hours (while the normal eggs float in such a solution for three days). If, however, the acetic acid solution is made up in $m/2$ NaCl (instead of distilled water) an exposure of the eggs of twenty minutes or more to the acid solution does not injure the membrane. Such eggs will float in 50 c.c. 3 *m* NaCl + 1 c.c. 2 1/2 *m* $CaCl_2$ three days or longer. By the same method it was ascertained that in the system H_2SO_4 — $m/2$ Na_2SO_4 the action of the acid was more effectively inhibited than in the system HCl — NaCl. From these experiments we are inclined to conclude that the increase in the permeability of the membrane for water and salt under the influence of acids is due to an alteration of the protein constituents of the membrane.

3. It was found that alcohols also increase the permeability of the membrane of the *Fundulus* egg for water (and possibly for salts). If eggs are put for sixty minutes into a grammolecular solution of methyl alcohol and then transferred to the test solution (50 c.c. 3 *m* NaCl + 2 c.c. 10/8 *m* $CaCl_2$) they will sink in less than eight hours (while the normal eggs float three days at the surface of such a solution). The relative efficiency of various alcohols for bringing about this increase in the permeability of the eggs was ascertained and it was found that each higher alcohol of the series is about three times as efficient as the preceding one. This is the well-known relation indicating effects on lipoids. The facts mentioned sub. 2 and 3 agree with the suggestion made by Natanson that cell membranes may be a mosaic of proteins and lipoids.

4. The increase in permeability caused by electrolytes and by alcohols is reversible if the eggs are put into sea water or into a $m/2$ solution of NaCl + KCl + $CaCl_2$ in the usual proportion. If the eggs are put into distilled water they may continue to live, and the fish may hatch, but the increase in permeability is not reversed. It can be shown that distilled water itself increases the permeability of the membrane very slowly.

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[4] *Pflüger's Archiv*, Bd. 75, p. 388, 1899.

[5] Beutner, *Biochemische Zeitschrift*, Bd. 39, 280, 1912.

VITAL STAINING OF CHROMOSOMES AND THE FUNCTION AND STRUCTURE OF THE NUCLEUS

ONE difficulty in studying protoplasm, particularly of living mitotic figures, is due to the slight differences in the refractive index of the various structures in the living cell. Up to the present, no satisfactory study has been made on the living chromosomes.

Our studies have been confined chiefly to the testes of the squash bug, grasshoppers and crickets, which are very favorable on account of the large size of their cells, and the clearness of the nuclear figures.

The testes were teased in Ringer's fluid and stained with Janus green (diethylsafraninazodimethylanalin) and studied in hanging drops in the Barber moist chamber. By variations in the concentration of the dye beautiful differential staining of the various cellular elements was obtained.

Masses of cytoplasmic granules varying in their position in the spermatogonia, sperma-

tocytes, spermatids and spermatozoa were stained a deep blue. The nuclear network of these cells and the chromosomes and spindle fibers, in all the division stages, were brought out with great sharpness by a somewhat longer application of the dye.

The separation of the dyad chromosomes in the metaphase figure of a primary spermatocyte of *Anasa* was observed. The transformation of anaphase figures of both spermatogonia and spermatocytes to telophase figures was easily followed.

When diethylsafraninazodimethylanalin is reduced the color changes from blue to red.

The possibility of studying nuclear reductions at once became apparent, when it was demonstrated that the stained chromosomes continued to live. By the use of appropriate methods we have been able to follow the relative rate of reduction in the nucleus and cytoplasm.

In the spermatid the first structure to turn red was found to be the "Nebenkern." Later all parts of the cell show this change. In the cells showing division figures the chromosomes and spindle fibers began to turn red while the remainder of the cell was still a deep slate blue. The same was found to be true for the nuclear network of resting cells. In the final stage of reduction, all stained cellular structures are red.

The colloidal structure of the resting and dividing nucleus was studied by means of dissections. The cells, in hanging drops, were dissected with Jena glass needles held in a three-movement pipette-holder. The needles were drawn in many cases to less than one half micron in diameter and the dissections were made under a 2 mm. Zeiss objective and Nos. 6 and 8 compensating oculars.

Resting and dividing spermatogonia, spermatocytes, spermatids and spermatozoa were dissected. Resting epithelial cells from the skin of the *Amblystoma* larva were also dissected.

The living cytoplasm of the spermatogonium, spermatocyte, spermatid and spermatozoon is extremely glutinous. It frequently adheres to the minute glass dissecting needle and a large portion of it can be drawn out into strands. This is particularly true of the spermatozoon. Dissections are greatly increased in difficulty, on account of this fact. Dying cells lose their viscidity and may be easily torn to pieces.

The masses of minute cytoplasmic granules, stained by Janus green, the "Nebenkern" and the middle piece of the spermatozoon, do not readily go into solution when dissected out in Ringer's fluid. Puncturing and tearing away parts of the cytoplasm of the spermatogonium and spermatocyte have no appreciable effect on the nucleus. When the cytoplasm or nucleus is punctured, the area immediately surrounding the needle stains a deep blue. If a portion of the nucleus be torn away the remainder does not collapse and gives no evidence whatsoever of loss of substance. The nuclear network can be torn out and is found to be a fairly concentrated, elastic gel, imbedded in a much more dilute viscous gel. Metaphase and telophase spindles neither collapse nor go into solution when freed from cytoplasm.

Single chromosomes were dissected out of cells in the prophase, metaphase and telophase stages. The chromosome is a fairly concentrated and somewhat refractive gel. It varies in elasticity in its different phases. A single metaphase chromosome was dissected out with its spindle fiber attached. The spindle fiber is a slightly refractive elastic gel and in the metaphase it seems to be continuous with the chromosome.

The nuclear network, spireme, spindle and chromosomes are imbedded in a dilute glutinous gel that is commonly invisible by the usual microscopical examination.

In two cases, while attempting to separate the daughter cells of a spermatocyte in telophase, a partial rapid reversal of the chemical and morphological changes occurring in cell-division was observed. In two seconds the daughter cells had fused and formed a single cell; the spindle fibers formed an irregular network in which the chromosomes were entangled. These observations seem to indicate that cell division is allied to contactility.

Resting epithelial cells from the skin of the *Amblystoma* larva were dissected for comparison. These cells are quite elastic. If a portion of the cytoplasm or nucleus be cut away, the remainder of the cell undergoes no demonstrable change in form. There is no evidence of a loss of substance from the nucleus when it is cut or torn. The nucleus in this cell is a quite concentrated gel. The intercellular matrix is non-viscous and highly elastic.

Extended studies in this field will be published later.

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EXPERIMENTS WITH DESICCATED THYROID, THYMUS AND SUPRARENALS[1]

THIS preliminary study of the effects of feeding the desiccated endosecretory organs was made on rabbits, guinea-pigs and fowls during June, July and August of the present year. The chief aim was to determine what proportion of the offspring of females given an excess of the dry substances were viable. The proportion of deformed offspring is not significant, but the action of the drugs on the fetuses and sucklings seems worthy of a brief note.

RESULTS IN THE PREGNANT RABBITS

Drug	Females	No. of Offspring	Deaths						Killed for Study on 4th Day
			At Birth	1 Day	2 Days	3 Days	23 Days	Living	
Thyroid..	4	24	2	10	3	6	1	2	
Thymus..	4	22	10			5		7	
Suprar...	1	11				2*		9	
Control...	1	10						6	4

From four to ten capsules (.76–1.9 gm.) of thyroid were given daily to rabbits during the last 20 days of their pregnancy, with no apparent symptoms of thyroidism. The offspring, however, either died at birth or during the first three days of lactation. Before their pregnancies it was found that from .38 gm. to .57 gm. of thyroid sufficed to produce extreme diarrhea and very rapid heart action; no exophthalmos developed. Weight decreased rapidly with .57 gm.

It was noted that if the offspring were not dead at birth and the heavy doses of thyroid were discontinued during lactation, the offspring lived.

In the case of one female of this thyroid group, preliminary feeding with thyroidectin had taken place until six days before parturition, when doses of thyroid increasing from .38 gm. to 1.52 gm. per diem were administered by the capsule method. The lactating young were killed on the third day of this treatment, although they had gained somewhat in weight during that time.

In the case of the thymus-treated females, the resistance to heavy doses (2.16–2.17 gm.) during the latter half of pregnancy also held. The offspring of three females were killed by the drug at an early age; one litter of the fourth succumbed at the third day of lactation, the other litter was born two days after the cessation of thymus feeding and though smaller than either of the two litters of the control rabbit in this series, lived. The effects of thymus on the adult females not in the later stages of pregnancy were similar to those in the non-pregnant females.

Unfortunately, but one of the suprarenal-fed rabbits gave birth during my period of experimentation. Her young were alive on the twenty-fifth day after birth, having grown much more rapidly than those of the control. Two of this litter were placed with one of the thymus females whose young had just died, on the day after birth, and two days later were dead. A third suckling from the suprarenal female was placed with the thyroid female which was receiving diminished doses during lactation, and this last adoption was successful also, but with the result that the stranger grew 5 gm. more in two days than a brother with the same initial weight in the home nest.

No discussion of these facts is needed; the table speaks for itself. These females were

[1] From the Station for Experimental Evolution, Carnegie Institution of Washington.